Monatshefte für Chemie 113, 399—407 (1982)

Monatshefte für Chemie
Chemical Monthly

The Complex Formation of Calcium with Aliphatic Dipeptides

Max J. A. Rainer and **Bernd M. Rode** *

Institute of Inorganic and Analytical Chemistry, University of Innsbruck, A-6020 Innsbruck, Austria

(Received 8 July 1981. Accepted 3 November 1981)

Dissociation constants for complexes of calcium with a series of dipeptides consisting of glycine, alanine, leucine and proline have been determined by *pH*-titrations. Two of these dissociation constants were checked by means of titration using a calcium sensitive electrode. The influence of the side chains on the stability of the complexes is discussed. Calcium forms only 1:1 complexes of the type $CaLH^+$ ($LH_2 = H_3\overset{+}{N}—CHR—CO—NH—CHR'—COO^-$) and probably also of the type $CaLH_2^{2+}$. The two PMR signals of the non-equivalent methylene protons of glycylglycine were measured with and without the addition of calcium chloride at various *pH*. On the basis of these results, possible structures of the complexes $CaLH^+$ are postulated.

(Keywords: Calcium-peptide complexes; Peptide complexes; Potentiometric titration; Stability constants)

Komplexbildung von Calcium mit aliphatischen Dipeptiden

Für eine Reihe Reihe von Dipeptiden, bestehend aus den Aminosäuren Glycin, Alanin, Leucin und Prolin, wurden Dissoziationskonstanten für deren Calcium-Komplexe mit Hilfe von *pH*-Titrationen bestimmt. Zwei dieser Dissoziationskonstanten wurden durch Messung mit einer Ca-sensitiven Elektrode überprüft. Der Einfluß der Seitenketten auf die Stabilität der Komplexe wird diskutiert. Calcium bildet nur 1:1 Komplexe der Form $CaLH^+$ ($LH_2 = H_3\overset{+}{N}—CHR—CO—NH—CHR'—COO^-$) und vielleicht auch der Form $CaLH_2^{2+}$. In einer H^1-NMR Untersuchung wurden die Signale der beiden nicht äquivalenten Methylengruppen von Glycylglycin gemessen, und zwar mit und ohne Zugabe von Calciumchlorid bei verschiedensten *pH*-Werten. Auf Grund dieser Ergebnisse werden mögliche Strukturen für die Komplexe vorgeschlagen.

0026-9247/82/0113/0399/$ 01.80

Introduction

As the investigation of metal-proteine complexes is quite complicated, short linear peptides are frequently used as model substances. Aliphatic dipeptides have 3 possible coordination abilities: N-terminal amino group, C-terminal carboxyl group and one peptide bond. While the interaction between dipeptides and transition metals has been studied extensively[1–4], only little is known about the complex formation of calcium with dipeptides[5,6].

Experimental

Chemicals: The concentration of the calcium chloride stock solution was determined by complexometric titration. The dipeptides listed in Table 1 were obtained from Sigma Chemical Co. and used without further purification.

pH-Titrations: All *pH*-measurements were performed using a Schott *pH*-meter CG 803 equipped with a standard glass electrode. Titrations were carried out at various metal/ligand ratios from 1:1.5 to 1:4. The concentration of calcium chloride was 2.012 m*M* in all titrations. The systems were titrated with 0.1 or 0.05 *M* NaOH. All investigations were carried out under nitrogen atmosphere at 20 °C and ionic strength of 0.2 *M* KCl. The stability constants were calculated using a Fortran program at the CDC computer of the University of Innsbruck.

Measurements with the Calcium Sensitive Electrode: The conditions were the same as described above. The concentration of the free calcium was measured with a Radiometer Selectrode which was connected to a Schott *pH*-meter CG 803.

PMR Measurements: PMR spectra were obtained by a Varian EM-360-L 60 MHz spectrometer at ionic strength of 0.2 *M* KCl in H_2O. The spectra were recorded at a sweep rate of 0.4 Hz/s and at a probe temperature of 30 °C, the sweep width was 120 Hz. The concentration of the dipeptide gly-gly was 0.1 *M*. Calcium chloride was added in equimolar amounts. 0.3 *M* KCl was added to solutions containing only gly-gly to give an ionic strength comparable to that of solutions containing calcium chloride. HCl or NaOH was added to bring the solutions to the desired *pH*. *TMA* (tetramethylammonium chloride) was used as internal standard. Chemical shifts are reported relative to the central resonance signal of the *TMA* triplet.

Results and Discussion

pH-Titrations

Calcium forms only weak 1:1 complexes with dipeptides. If LH_2 denotes the zwitterionic form, the following reactions can be defined:

$$K1\text{: } LH_2 + H^+ \rightarrow LH_3^+$$
$$K2\text{: } LH_2 \rightarrow LH^- + H^+$$
$$K3\text{: } CaLH^+ \rightarrow Ca^{2+} + LH^-$$

Table 1. *Stability constants of calcium/dipeptide complexes (pK1, pK2, pK3, dpK3 notation see text; superfixes correspond to references)*

)eptide	$pK1$[4]	$pK1^{Lit.}$	$pK2$[4]	$pK2^{Lit.}$	$pK3$	$pK3^{Lit.}$	d$pK3$
-gly	—3.18	—3.17[7]	8.25	8.13[7]	2.04	1.24[6]	0.23
		—3.23[8]		8.19[8]			
-*d,l*-ala	—3.19	—3.17[7]	8.40	8.20[7]	2.02	—	0.26
		—3.15[8]		8.23[8]			
-*d,l*-leu	—3.20	—3.28[8]	8.37	8.23[8]	1.93	—	0.18
-*l*-pro	—2.93	—2.97[9]	8.77	8.48[9]	2.57	—	0.10
ala-gly	—3.22	—	8.33	8.27[8]	1.87	0.66[6]	0.39
leu-gly	—3.20	—3.28[8]	8.24	8.07[8]	1.74	0.70[6]	0.22
ro-gly	—3.05	—3.19[9]	9.15	8.98[9]	1.50	—	0.29
ala-*d,l*-ala	—3.18	—3.08[7]	8.39	8.26[7]	2.15	—	0.27
ala-*d,l*-leu	—3.15	—	8.36*	8.32[4]	1.74	—	0.30
ro-*l*-ala	—3.20	—	9.19	—	1.77	—	0.48
ro-*l*-leu	—3.21	—	9.16	—	2.22	—	0.18

[4] 0.2 *M* KCl, 20 °C, used in this work.
[6] Saturated $Ca(IO_3)_2$, 25 °C.
[7] 0.2 *M* KCl, 25 °C.
[8] 0.1 *M* KCl, 25 °C.
[9] 0.16 *M* KNO_3, 25 °C.
* Determined and used in this work, 0.2 *M* KCl, 20 °C.

The simulation of the titration curves including the formation of further complex species did not improve the quality of the simulation. The constants for the protolysis of the pure dipeptides were mostly taken from literature[4]. Table 1 lists the dipeptides, their constants for the protolysis and the dissociation constants of the calcium complexes. Some of these dipeptides were used in the *d,l*-form; therefore the calculated dissociation constants must be regarded as mean values for all stereoisomers present[3]. d$pK3$ serves as a measure for the significance of the constant $pK3$. Changing the constant $pK3$ by $\pm$ d$pK3$ leads to an increase of $\sum_i (NaOH_i^{theoret.} - NaOH_i^{calc.})^2$ by a factor 2.

Because of the low stabilities of the complexes and the relatively high values of d$pK3$ it is difficult to discuss the influence of the side chains on the stability. The larger R and R', the lower seems to be the stability of the complexes; one can deduce from this fact, that both amino acids are involved in the complexation of the calcium ion. Proline is an exeption to this rule, presumably because of its rigid ring

system. Gly-pro forms the most stable complex with calcium, whereas pro-gly has the lowest stability constant in this series. *Davies* and *Waind*[6] have suggested some values for the stability constants. These values were determined by measurement of the solubility of $Ca(IO_3)_2$ in solutions containing dipeptides and were corrected by activity coefficients. This correction might be one of the reasons that their values deviate from those presented in this paper.

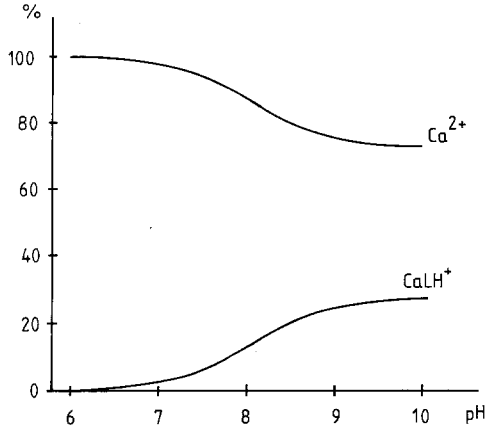

Fig. 1. Distribution of species as a function of pH. Concentration is given in percent of the total metal concentration. Total concentration of calcium: 0.002 M; total concentration of gly-gly: 0.004 M

Figs. 1 and 2 illustrate the species distribution depending on pH. The maximum concentration of $CaLH^+$ at higher pH-values depends only on $pK3$ because the dipeptide is deprotonated; at medium pH range a low $pK2$ value also favours complex formation.

Measurements with the Calcium Sensitive Electrode

The free calcium concentration can be computed for every point of the titration curves if the constants $pK1$, $pK2$ and $pK3$ are fixed. The free calcium concentration can also be measured by the aid of a calcium sensitive electrode. Therefore the pH-titration method can be checked by this method. Investigations were carried out with two systems as shown in Figs. 3 and 4. Figs. 3 and 4 illustrate that results being identical within the experimental error ($dpK3$) can be obtained by both methods A and B.

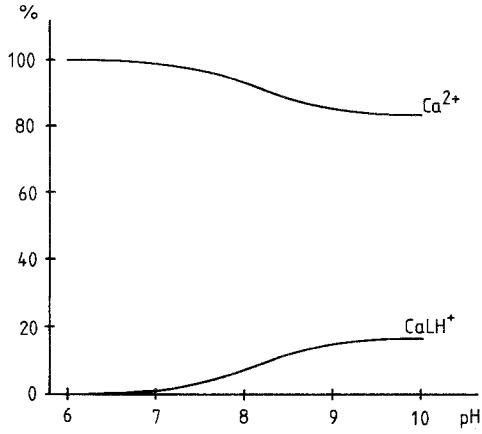

Fig. 2. Distribution of species as a function of pH. Concentration is given in percent of the total metal concentration. Total concentration of calcium: 0.002 M; total concentration of *d,l*-leu-gly: 0.004 M

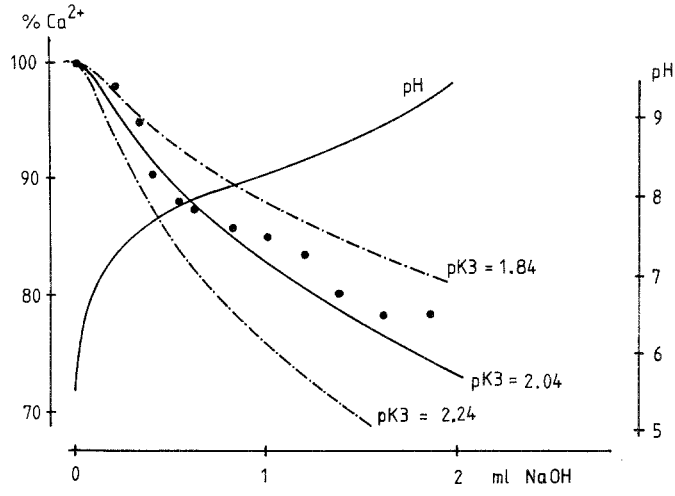

Fig. 3. Titration curve (pH) for the system calcium + gly-gly. Total concentration of calcium: 2.012 mM; total concentration of gly-gly: 4.058 mM; ionic strength: 0.2 M KCl; temperature: 20 °C; 50 ml solution were titrated with 0.1 M NaOH; ——— free calcium concentration computed with the constants in Table 1; —·—·— free calcium concentration computed at $pK3 \pm 0.2$; ● free calcium concentration determined by the aid of a calcium sensitive electrode

PMR Measurements

Gly-gly has two non-equivalent methylene groups:

$$\underset{\beta}{H_3\overset{+}{N}-CH_2}-CO-NH-\underset{\alpha}{CH_2}-COO^- \text{ (zwitterionic form)}$$

The two signals appear as broad singlets because of exchange effects. The influence of the addition of calcium chloride on the

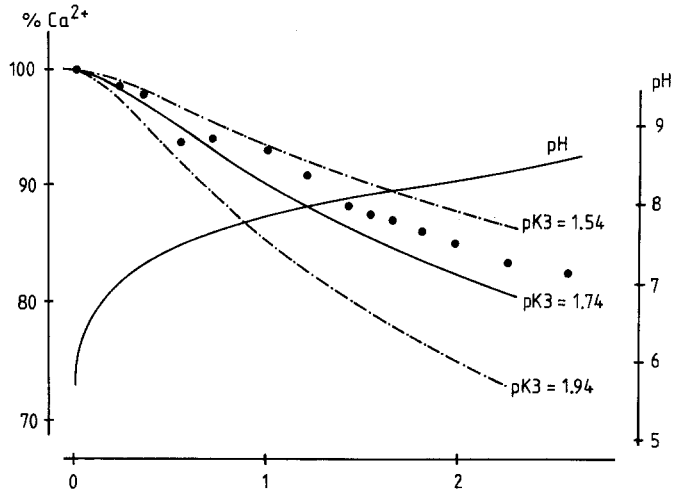

Fig. 4. Titration curve (pH) for the system calcium + d,l-leu-gly. Total concentration of calcium: 2.012 mM; total concentration of d,l-leu-gly: 8.002 mM; ionic strength: 0.2 M KCl; temperature: 20 °C; 50 ml solution were titrated with 0.1 M NaOH; ——— free calcium concentration computed with the constants in Table 1; —·—·— free calcium concentration computed at $pK3 \pm 0.2$; ● free calcium concentration determined by the aid of a calcium sensitive electrode

chemical shift to gly-gly as well as the influence of pH on both calcium free and calcium complexed gly-gly should serve as a powerful tool for the interpretation of the complex structures. The assignment of the signals is facilitated by previous works on gly-gly and its complexes with transition metals[10,11]. Figs. 5 a, b shows the distribution of the species depending on pH at conditions described in the experimental section on PMR measurements. Fig. 6 illustrates the chemical shift of the methylene protons of gly-gly relative to TMA. All signals appear at lower field than the TMA standard signal.

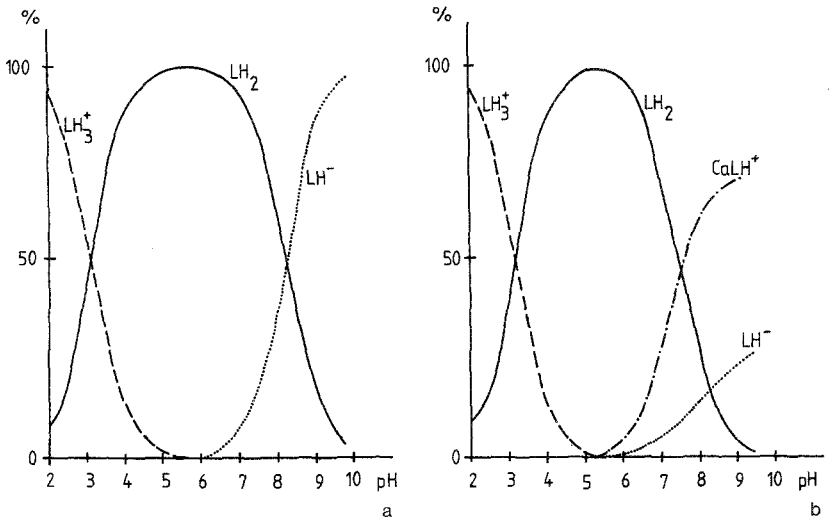

Fig. 5. *a* Distribution of species as a function of *pH*. Concentration is given in percent of the total gly-gly concentration; total concentration of gly-gly: 0.1 *M*. *b* Distribution of species as a function of *pH* after addition of 0.1 *M* calcium chloride

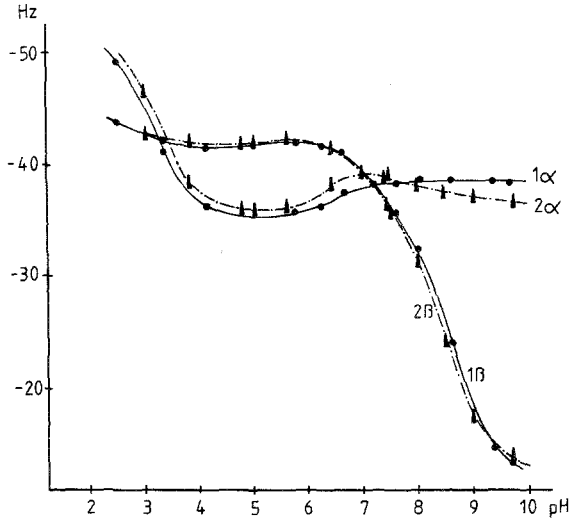

Fig. 6. Chemical shift of the α and β methylene protons relative to *TMA* as a function of *pH*; *1* — ●—● — pure gly-gly; *2* —▲·—·▲·—· gly-gly + calcium chloride

Pure gly-gly: The significant downfield shift of the α methylene proton signal at $pH = pK1$ is apparently due to the protonation of the carboxylate group. In this pH range the shift of 1 β is relatively small. The sigmoide shape of the curve 1 β at $pH = pK2$ should occur because the amino group loses a proton. In this pH range 1 α slightly shifts downfield.

Gly-gly + calcium chloride: The formation of the calcium complex scarcely influences the shift of the β methylene protons. It is known that complex formation with the amino group would cause a consider-

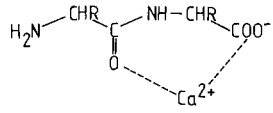

Fig. 7. Postulated structure of the complexes $CaLH^+$

able upfield shift[10–12]. This observation leads to the conclusion that the amino group is not involved in the formation of the complex. Thus the carboxylate group and/or the peptide bond may be regarded as possible binding abilities. The assumption of pure carboxylate interaction would result in a downfield shift of 2 α; actually complex formation leads to an upfield shift. It is to be assumed therefore that a chelate complex is formed via the peptide oxygen and the carboxylate group. The fact that in the medium and low pH range 2 α deviates from 1 α by showing a small shift to the lower field can be explained by a partial formation of the weak complex $CaLH_2^{2+}$ ($H_3\overset{+}{N}—CH_2—CO—NH—CH_2—COO^- \cdots Ca^{2+}$)[10,13], which is not detectable by the method of pH-titrations, as no protons are set free upon complex formation.

Acknowledgement

Financial support by the "*Fonds zur Förderung der wissenschaftlichen Forschung*", Vienna, Austria (Project No. 3725), is gratefully acknowledged.

References

1 *Li N. C., Chen M. C.*, J. Amer. Chem. Soc. **80**, 5678 (1958).
2 *Kollmann J., Hoyer E.*, J. Prakt. Chem. **316**, 119 (1974).
3 *Nakon R., Angelici R. J.*, J. Amer. Chem. Soc. **96**, 4178 (1974).
4 *Kittl W. S., Rode B. M.*, Inorg. Chim. Acta **55**, 21 (1981).
5 *Märki W., Opplinger M., Schwyzer R.*, Helv. Chim. Acta **60**, 807 (1977).

[6] *Davies C. W., Waind G. M.*, J. Chem. Soc. **1950**, 301.
[7] *Nagypal I., Gergely A.*, J. Chem. Soc. Dalt. Trans. **11**, 1104 (1977).
[8] *Feige P., Mocker D., Dreyer R., Münze R.*, J. Inorg. Nucl. Chem. **35**, 3269 (1973).
[9] *Koltun W. L., Fried M., Gurd F. R. N.*, J. Amer. Chem. Soc. **82**, 233 (1960).
[10] *Rabenstein D. L., Libich S.*, Inorg. Chem. **11**, 1960 (1972).
[11] *Tewari K. C., Li N. C.*, Trans. Farad. Soc. **66**, 2069 (1970).
[12] *Jezowska-Trzebiatowska B., Latos-Grazynski L., Koslowski H.*, J. Inorg. Nucl. Chem. **39**, 1269 (1977).
[13] *Nag K., Banerjee P.*, J. Inorg. Nucl. Chem. **36**, 2145 (1974).